NUCLEAR MAGNETIC RESONANCE AND CALORIMETRIC STUDY OF THE STRUCTURE, DYNAMICS, AND PHASE BEHAVIOR OF URANYL ION/DIPALMITOYLPHOSPHATIDYLCHOLINE COMPLEXES

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ABSTRACT The interaction of UO_2^{2+} with dipalmitoylphosphatidylcholine (DPPC) has been studied as a function of temperature and composition using nuclear magnetic resonance (NMR) spectroscopy, differential scanning calorimetry (DSC), and monolayer studies. Computer simulations of the ^{31}P-NMR powder spectra of DPPC dispersions in the presence of various concentrations of UO_2^{2+} are consistent with the binding stoichiometry of $[UO_2^{2+}]/[DPPC] = 1:4$ at $[UO_2^{2+}]/[DPPC] < 0.3$. This complex undergoes a phase transition to the liquid crystalline phase at $T_m' = 50 \pm 3$°C with a breadth $\Delta T_m' = 7 \pm 3$°C. This broad transition gradually disappears at higher UO_2^{2+} concentrations, suggesting the presence of yet another UO_2^{2+}/DPPC complex (or complexes) whose NMR spectra are indistinguishable from those of the 1:4 UO_2^{2+}/DPPC species. The temperature-dependent ^{13}C powder spectra of 2(1-^{13}C) DPPC dispersions in the presence of 1.2 mol ratio of UO_2^{2+} show that this higher order complex (complexes) also undergoes a phase transition to the liquid crystalline state at $T_m' \pm = 58 \pm 3$°C with a breadth $\Delta T_m'' = 15 \pm 5$°C. The NMR spectra indicate that exchange among these various UO_2^{2+}/DPPC complexes is slow. In addition, computer simulations of the ^{31}P-, ^{13}C-, and ^{2}H-NMR powder spectra show that axial diffusion of the DPPC molecules about their long axes is quenched by addition of UO_2^{2+} and acyl chain isomerization is the dominant motional mode. The isomerization is best described as two-site hopping of the >C-D bond at a rate of $\sim 10^6$ s^{-1}, a motional mode which is expected for a kink diffusion.

INTRODUCTION

The interaction of cations with phospholipids plays an important role in biological function (Hauser and Philip, 1979). Despite numerous studies on this subject, many of the details concerning the mechanism of cation binding and of the molecular events caused by the binding are still relatively unclear. For example, it is known that divalent ions such as Ca^{2+}, Cd^{2+}, and Mg^{2+} bind tightly to charged species such as phosphatidic acids (PA) and phosphatidylserine (PS) and raise the transition temperatures of these lipids dramatically (Papahadjopoulos et al.; Liao and Prestegard, 1979; Browning and Seelig, 1980; Hauser and Shipley, 1984), and similar effects are observed for the Li^+ in PS dispersions (Hauser and Shipley, 1984). In contrast, other monovalent cations, Na^+, K^+, and NH_4^+, produce only small perturbations to PS phase transitions. Furthermore, these same cations, as well as divalent ions, apparently produce relatively minor effects on neutral lipids such as phosphatidylcholines (PCs) (Brown and Seelig, 1977; Akutsu and Seelig, 1981). However, one cation, uranyl ion (UO_2^{2+}) interacts quite strongly with dipalmitoylphosphatidylcholine (DPPC) and produces dramatic changes in the structure and dynamics of the lipid molecules. In the gel phase UO_2^{2+} immobilizes the PO_4 part of the head group as judged by the ^{31}P-NMR spectra and the transition temperature is raised by ~20°C (Chapman et al., 1974; Shah, 1968; Rajan et al., 1981). In the high temperature L_α-like phase the chain splitting displays an ordering profile similar to that produced by cholesterol (Oldfield et al., 1978). In addition, Parsegian, et al. (1981) have demonstrated that addition of UO_2^{2+} to gel state DPPC induces an $L_\beta' \rightarrow L_\beta$ transition, the hydrocarbon chains become parallel to the bilayer normal. Furthermore, these workers found that the change of tilt occurs between 0–10 mol % of UO_2^{2+} and that the changes are highly cooperative. More recently, Caffrey et al. (1987) reported an x-ray diffraction study and concluded that uranyl acetate induces gel phase formation in chromaffin granule lysed membrane. The liquid crystal to gel phase transition temperature in this biological membrane was reported to be between 2° and 10°C. Although uranyl ion has no natural biological significance, the wide usage of uranyl acetate as a stain in electron microscopy provide the impetus for studying the interaction of uranyl ions with membrane. Here, we report nuclear magnetic resonance

(NMR), differential scanning calorimetry (DSC), and monolayer studies of the molecular structure and dynamics of the UO_2^{2+}/DPPC complex. We have found that UO_2^{2+} binds to the phosphodiester group in a stoichiometry of UO_2^{2+}/lipid = 1:4 and that this binding of the ion dramatically reduces the axial diffusion rates of the lipid molecules. This results in ^{31}P powder lineshapes similar to those observed in the rigid lattice regime i.e., in dry lipid powders or at low temperatures. ^{13}C spectra of (1-^{13}C)DPPC are broadened by addition of the ion, and ^2H chain spectra are similar. At UO_2^{2+}/DPPC = 1.2:1, the gel-liquid crystalline transition temperature increased by ~20°C.

MATERIALS AND METHODS

Lipid Synthesis

[1-^{13}C] palmitic acid was synthesized by reaction of a long-chain bromide with $^{13}C{=}N$ and subsequent hydrolysis to the fatty acid or was purchased from Cambridge Isotopes, Woburn, MA. The synthesis of ^2H-labeled fatty acids is discussed elsewhere (Das Gupta et al., 1982). Phosphatidylcholine ^{13}C labeled at the carbonyl carbon of the sn-2 chain, 2-[1-^{13}C]-DPPC, and ^2H labeled at the 6 position of sn-2 chain [2(6,6-d_2)DPPC] was prepared by acylation of the corresponding lysophosphatidylcholine with the fatty acid anyhydride by using N,N-dimethyl-4-aminopyridine as a catalyst (Gupta et al., 1977). Purity was routinely checked with TLC on silica gel plates.

NMR Spectroscopy

Solid state NMR spectra were obtained on a home-built pulse spectrometer operating at 119 MHz for ^{31}P, 73.9 MHz for ^{13}C, and at 45.1 MHz for ^2H, corresponding to an ^1H frequency of 294 MHz. In most cases, the ^{13}C and ^{31}P spectra were recorded with a Hahn echo which circumvents problems with receiver overload and acoustic probe ringing and thus yields powder spectra free of distortions (Griffin, 1981; Rance and Byrd, 1983). In many cases, the spectral effects observed are strongly temperature-dependent. Consequently, we were particularly careful to keep the ^1H decoupling power at the minimum level necessary to obtain well-resolved lines and thus, insofar as possible, to avoid sample heating. Recycle delays in the ^{13}C experiments were typically 5 and 10 s for ^{31}P experiments which helped to further minimize this problem. The ^{13}C $\pi/2$ pulse was $\simeq$3 μs. All ^{13}C shifts are referenced to external benzene and ^{31}P shifts to external 85% phosphoric acid. ^2H NMR spectra were obtained with a two-pulse quadrupole echo (Solomon, 1958; Davis et al., 1976) using a $\pi/2$ pulse of 1.7 μs. Phase cycling was used in both echo experiments, and in both cases, quadrature phase detection was employed.

Samples for NMR experiments generally consisted of ~50 mg of lipid dispersed in an equal amount of deuterium depleted H_2O (Aldrich Chemicals Co., Milwaukee, WI) containing appropriate amount of $UO_2(NO_3)_2$ or uranyl acetate. The stock solutions of $UO_2(NO_3)_2$ and $UO_2(CH_3COO)_2$ were neutralized with NaOH until first precipitates appeared. Samples for the NMR experiments were sealed in 7-mm glass tubes. TLC's were generally taken after completion of the experiments to check for decomposition.

Differential Scanning Calorimetry

Calorimetry was performed using a home-built adiabatic scanning calorimeter (Schupp, 1980) with a sample volume of 2.8 ml. The samples were usually scanned three to four times using a heating rate of 0.5 deg/min. In some cases, the second scan differed from the first, indicating incomplete equilibration. The consecutive scans such as are shown in Fig. 5, exhibit better reproducibility. The samples used for the calorimetric experiments contained 2–6 mg/ml of lipid and were prepared by suspending the required amount of DPPC in bi-distilled water, and adding an aliquot of a concentrated $(UO_2)(NO_3)_2$ solution to give the appropriate UO_2^{2+}/DPPC ratio. Subsequently the suspension was sonicated at 55°C for 2 to 3 min using a MSE Ultrasonic Disintegrator with a 1/8 inch titanium tip. After adjustment of the pH value 5 to 6 the dispersion was equilibrated at 65°C for 1 to 2 h before placing it in the calorimeter cell.

Monolayer Experiments

Monolayer experiments were performed as described before, using a commercial MGW Lauda film balance with an automatic pressure control system for measuring isobars (Blume, 1979). Isobars at a pressure of 25 dynes/cm were recorded at increasing temperature using a heating rate of ~1.4 deg/min. The isobars shown in Fig. 7 are averaged from two to three different runs.

RESULTS

NMR Experiments

Shown in Fig. 1 *a* are the proton-decoupled ^{31}P NMR spectra of DPPC/50 wt % H_2O at 22°C and in the presence of various molar ratios of UO_2^{2+}. In the absence of UO_2^{2+}, the ^{31}P spectrum is axial symmetric with breadth ~60 ppm ($\sigma_{11} = -40$ ppm, $\sigma_{22} = \sigma_{33} = 20$ ppm relative to H_3PO_4) which is characteristic of an axially diffusing DPPC molecule. At $[UO_2^{2+}]/[DPPC] = 0.5$, the spectrum becomes axially asymmetric of breath ~106 ppm ($\sigma_{11} = -48$ ppm, $\sigma_{22} = -18$ ppm, $\sigma_{33} = 58$ ppm). Further increases in UO_2^{2+} concentration produce no additional change in the ^{31}P spectrum. Shown in Fig. 1 *b* are the computer simulations of the experimental spectra obtained by adding appropriate fractions of the pure DPPC spectrum (*top*) and the spectrum at $[UO_2^{2+}]/[DPPC] = 0.5$ (*bottom*) of Fig. 1 *a*. The good agreement between the experimental and simulated spectra suggests the presence of two types of lipids; free and UO_2^{2+}-bound, and that exchange between these is slow.

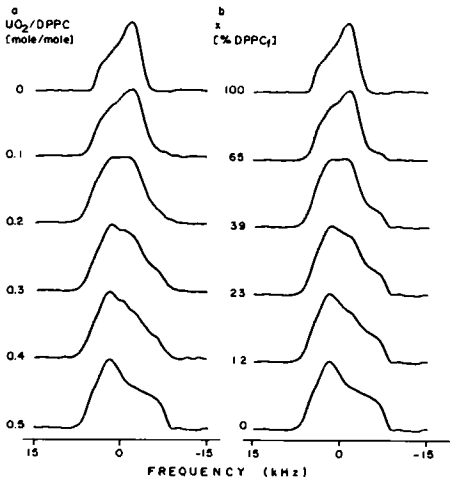

FIGURE 1 Proton-decoupled ^{31}P-NMR spectra of DPPC/50 wt % H_2O in the presence of various amounts of UO_2^{2+} at 22°C. (*a*) Experimental spectra; (*b*) simulated spectra. Simulated spectra were obtained by adding appropriate fractions of pure DPPC spectrum (*top*) with that of the spectrum at 0.5 mol fraction of UO_2^{2+} (*bottom*).

Fig. 2 shows the ^{13}C NMR spectra of 2(1-^{13}C)DPPC/UO_2^{2+} (1:1.2 mol ratio) dispersed in 50 wt % H_2O at various temperatures (Fig. 2 *a*) and the corresponding computer simulations which were obtained by adding appropriate fractions of the spectra at 40° and 64°C (Fig. 2 *b*). Below 40°C, the spectrum is slightly axially asymmetric with a breadth ~130 ppm, while above 64°C, only an isotropic line of linewidth ~300 Hz was observed. Between these two extremes, composite spectra are observed which are well represented as a simple superposition of the 40° and 64°C components. This observation directly indicates that exchange between the two components is slow on the ^{13}C time scale, a point which will be discussed further below. The 130 ppm breadth observed at <40°C is almost equal to that found in dry DPPC powders (Wittebort et al., 1982).

Fig. 3 shows the ^2H quadrupole NMR spectra of 2(6,6-^2H)DPPC/UO_2^{2+} (1:1.2 mol ratio) in 50 wt % H_2O (deuterium depleted) at various temperatures (Fig. 3 *a*) and the corresponding computer simulations (Fig. 3 *b*). In this case, the ^2H NMR spectra at intermediate temperatures (40°–60°C) cannot be simulated simply by adding appropriate fractions of the broad and the narrow components. To obtain satisfactory simulations, it was necessary to broaden the narrow L_α-like component in the low temperature spectra. The broad L_β-like component of the spectrum was obtained by allowing the deuteron to jump among the tetrahedral sites of population ratios 0.63:0.3:0.07 and jumping rates from site 1 to 2, $k_{12} = 3.1 \times 10^7\ s^{-1}$, from site 1 and 2 to site 3, $k_{13} = k_{23} = 1.95 \times 10^6\ s^{-1}$ (Wittebort et al., 1987). The quadrupole coupling constant was $(e^2qQ/h) = 167$ kHz. The narrow component was assumed to arise from a **C-D** vector executing fast axial diffusion and *gauche-trans* isomerization which leads to a quasistatic ^2H spectrum with $\Delta\nu_Q = 34$ kHz. These two components were then superimposed to obtain a best fit to the spectrum. With the exception of the 40°C spectrum, the fits are quite good. The fraction of L_α component, as obtained from these computer simulations, and from fits of the ^{31}P and ^{13}C spectra is shown in Fig. 4 as a function of temperature.

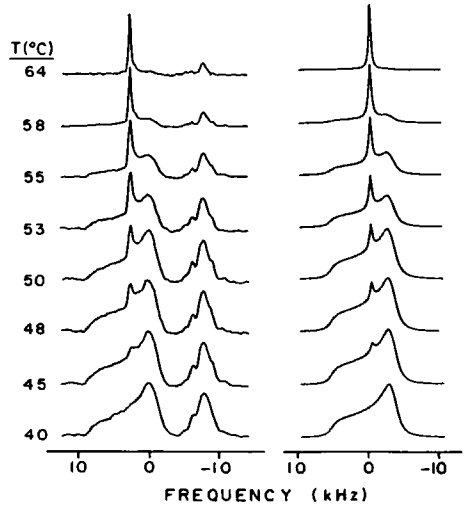

FIGURE 2 ^{13}C-NMR spectra of 2(1-^{13}C)DPPC/1.2 UO_2^{2+} at various temperatures (*left*). Also shown (*right*) are computer simulations performed by addition of appropriate proportions of the isotropic spectrum (*top*) to the immobilized ^{13}C spectrum (*bottom*).

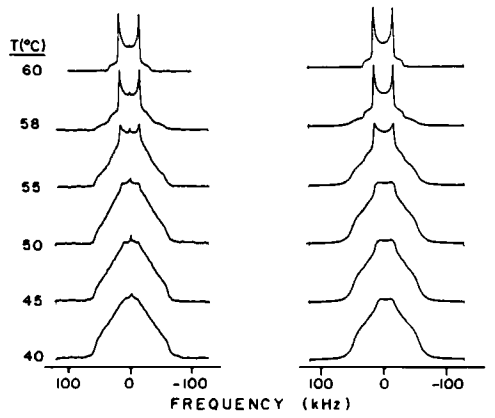

FIGURE 3 (*a*) Temperature-dependent ^2H-NMR sepctra of 2(6,6-$^2H_2$)DPPC/50 wt % H_2O in the presence of 1.2 mol ratio of UO_2^{2+}. (*b*) Computer simulation of *a* assuming a two-site tetrahedral jump model. See text for details.

Calorimetric Experiments

Fig. 5 shows the calorimetric scans of DPPC at various UO_2^{2+}/DPPC ratio's as indicated. The distinctive feature of these scans is the disappearance of the sharp transition at 41.5°C and the pretransition at ~35°C. Instead, at $1.0 \geq [UO_2^{2+}]/[DPPC] \geq 0.5$, a broad, relatively featureless transition is observed which is shifted to higher temperature and is centered at $T_m \simeq 50 \pm 2$°C with $\Delta T_m = 7 \pm 3$°C. At intermediate UO_2^{2+} concentrations, the intensity of the broad component decreases with increasing UO_2^{2+}/DPPC ratio's and is barely detectable at $[UO_2^{2+}]/[DPPC] = 1.0$. This decrease in intensity suggests the formation of a second UO_2^{2+}/DPPC complex whose transition cannot be detected with our differential scanning calorimeter within the temperature range of 30° to 70°C. In Fig. 6 is shown the variation of the transition enthalpy obtained by integration of the broad and narrow peaks in Fig. 5, including the pretransition with the UO_2^{2+}/DPPC ratio. An almost linear decrease of ΔH was obtained.

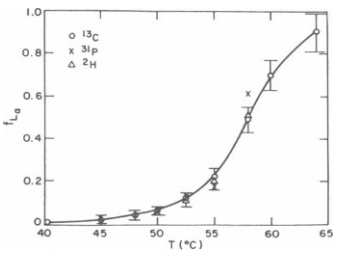

FIGURE 4 Fraction of the L_α-component (f_{L_α}) as a function of temperature obtained from computer simulations of the corresponding ^{13}C-, ^{31}P-, and ^2H-NMR spectra.

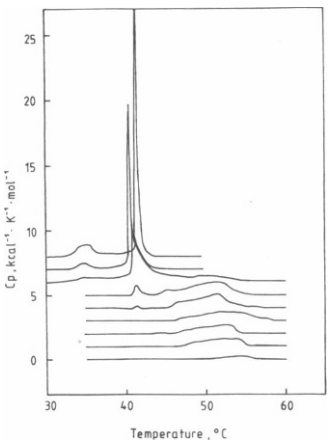

FIGURE 5 Calorimetric scans of UO_2^{2+}/DPPC dispersions at various $[UO_2^{2+}]/[DPPC]$ ratios. Notice that the pre and main transitions are broadened and shifted to higher temperatures with increasing UO_2^{2+} concentration. Traces from top to bottom correspond to UO_2^{2+}/DPPC ratios of 0, 0.10, 0.20, 0.30, 0.40, 0.50, 0.70, 0.80, and 1.0.

Monolayer Experiments

Fig. 7 shows isobars (P = 25 dyne/cm) of DPPC monolayer on pure water and on $(UO_2)(NO_3)_2$ solution as subphase. With increasing UO_2^{2+} concentration the temperature for the phase transition from the liquid-condensed to the liquid-expanded phase increases. At the same time, UO_2^{2+} binding to the DPPC headgroups reduces the molecular area in the liquid condensed phase at 22°C from 49 to 45Å^2. In the liquid-expanded phase the condensation is even more pronounced with an area change from 67 to 57 Å^2/molecule at 58°C. However, with increasing UO_2^{2+} concentration, the area change at the transition does become smaller.

DISCUSSION

To understand the effect of cations on membrane it is essential to obtain information concerning the binding stoichiometry, the structure of the ion-lipid complex, and the effect of ion binding on molecular dynamics. We now discuss our data in terms of these three aspects of membrane-cation interaction.

To determine the binding stoichiometry at a particular temperature we have obtained fractions of free and UO_2^{2+}-bound lipid components as a function of UO_2^{2+} concentration through computer simulation of the ^{31}P NMR spectra at 22°C. This is possible because of the slow exchange between the two components which allows unambiguous and accurate simulation of the ^{31}P spectra, examples of which are shown in Fig. 1. Assuming n equivalent noninteractin DPPC binding sites per uranyl ion, then for each site M.

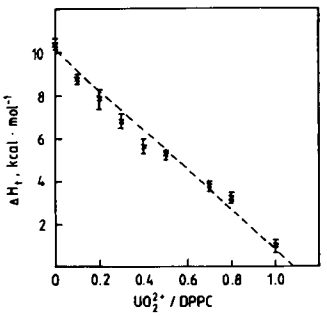

FIGURE 6 Variation with UO_2^{2+} content of the transition enthalpies obtained by integration of the broad and narrow peaks in the calorimetric scans shown in Fig. 5.

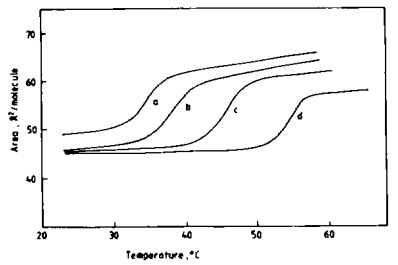

FIGURE 7 Isobars (p = 25 dyn/cm) of monomolecular films of DPPC on (a) pure water, (b) 0.1 mM $UO_2(NO_3)_2$, (c) 1.0 mM $UO_2(NO_3)_2$, and (d) 10 mM $UO_2(NO_3)_2$ solution as subphase.

$$\text{DPPC} + M \rightleftarrows M(\text{DPPC}) \tag{1}$$

and

$$K = \frac{[M(\text{DPPC})]}{[\text{DPPC}]_f[M]_f}, \tag{2}$$

where K is the association constant, square brackets represent the molar concentration and f stand for species in the free form. Rearranging Eq. 2 and using the fact that the concentration of DPPC binding site is n times the uranyl ion concentration, we obtain.

$$\frac{\overline{U}}{[\text{DPPC}]_f} = K(n - \overline{U}), \tag{3}$$

where $\overline{U} = [\text{DPPC}]_b/[UO_2^{2+}]_t$ is the ratio of bound DPPC concentration and the total uranyl ion concentrations. Fig. 8 shows a plot of $\overline{U}/[\text{DPPC}]_f$ vs. $\overline{U}$, which is the standard Scatchard plot (Marshall, 1978). A straight line is obtained, which confirms the validity of Eq. 3, which has a slope of $-K$ and intercept the horizontal axis at $\overline{U} = n$. Since the lipid dispersion is not a real solution, the lipid concentration cannot be expressed in molar concentration units. We have used fractional population of free and bound species for $[\text{DPPC}]_f$ and $[\text{DPPC}]_b$, respectively. For $[UO_2^{2+}]_t$ the molar fractions are used. With these arbitrary concentration units the association constant obtained from the slope of Fig. 8 is only proportional to the actual association constant. However, the number of binding sites per uranyl ion, n, can be obtained from the intercept to the horizontal axis and is independent of concentration units. The best least square fit of these data yields $n = 4.3 \pm 0.5$.

This stoichiometry of 4:1 DPPC/UO_2^{2+} complex is consistent with the DSC scans as shown in Fig. 5. In the limit of an infinite association constant, each UO_2^{2+} molecule is expected to bind four DPPC molecules. Therefore,

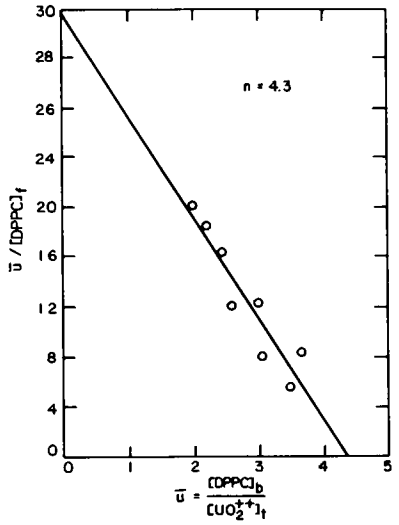

FIGURE 8 Scatchard plot of the ^{31}P-NMR spectra of DPPC/50 wt % H_2O at various $UO_2(NO_3)_2$ concentrations. Spectra were taken at 22°C. Experimental points were obtained from computer simulations of the corresponding ^{31}P-NMR spectra. The best-fit of these points yield the number of DPPC bound per UO_2^{2+} of 4.3.

no free DPPC molecule will be observed at $[UO_2^{2+}]/[DPPC] = 0.25$. The small sharp component remaining at $[UO_2^{2+}]/[DPPC] = 0.30$ can be attributed to the finite binding constant which cannot be estimated with our method. The broad component centered at ~50°C can be attributed to the melting of this 1:4 $[UO_2^{2+}]/[DPPC]$ complex. In addition, the calorimetric experiments suggest the presence of a higher-order complex which exhibits a decreasing enthalpy of transition at $[UO_2^{2+}]/[DPPC] > 0.5$. At $[UO_2^{2+}]/[DPPC] > 1.0$, no transition can be detected. The presence of this higher order complex is supported by the temperature-dependent ^{31}P, ^{13}C, and ^{2}H NMR spectra (Figs. 1–3). The result of computer simulations of these NMR spectra is presented in Fig. 4 which shows the fraction of the mobile component as a function of temperature. The $[UO_2^{2+}]/[DPPC]$ complex transforms from a relatively immobilized molecule (this will be discussed further later) to a relatively mobile and presumably liquid crystalline-like molecule. The transition curve is sigmoidal and takes place between ~50°–65°C with the midpoint occurring at T_m' ~58°C, compares with 50°C for the 1:4 $[UO_2^{2+}]/[DPPC]$ complex. The higher transition temperature and the broader temperature range for the transitions (~15°C) clearly suggest the presence of a higher order complex. The inability to detect this higher order transition with our calorimeter can partially be attributed to the broader transition, with less cooperativity and to the smaller transition enthalpy. Moreover, since the NMR spectra of $[UO_2^{2+}]/[DPPC]_4$ are indistinguishable from those of the higher order complex, we cannot deduce the binding stoichiometry. We would like to mention that our calorimetric measurements reveals much more complex behavior than was previously reported by Chapman et al. (1974). A discrepancy which may be due to the different method of sample preparation and differences in total lipid concentration. As pointed out in the experimental procedure section, it is important to control the solvent pH and to equilibrate the sample for a relatively long period.

From a comparison of the infrared spectra of egg-lecithin and UO_2^{2+}-lecithin complex, Chapman and coworkers (Chapman, et al., 1974) have shown that the strong binding of the ion is specific to the phosphate groups, an idea which is supported by our ^{31}P NMR data (Fig. 1). The ^{31}P chemical shift powder spectrum of an immobilized PO_4 in the phosphodiester linkage is axially asymmetric with a breadth of ~180 ppm (Herzfeld, et al., 1978). In DPPC/50 wt % H_2O dispersion, the molecule undergoes rapid axial diffusion and the spectrum is motionally averaged to an axially-symmetric spectrum of breadth ~60 ppm (Griffin, 1981). Upon addition of 1.2 mol ratio of UO_2^{2+}, an axially-asymmetric pattern of breadth ~160 ppm is observed. Such a spectrum of intermediate breadth can be obtained through motional averaging mechanism or through direct binding of uranyl ion to the phosphodiester group. To obtain an axially asymmetric spectrum through motional averaging the motional process must have twofold or lower symmetry such as two-site jump or libration or a three-site jump with unequal population (Huang et al., 1980). The ^{13}C spectrum in Fig. 2 provides evidence that the spectral change is caused by a direct binding of uranyl ion to the phospho- iester group. At 10°C the ^{13}C spectrum of the sn-2 $C=O$ carbon is axially-asymmetric and of ~140 ppm breadth in the anhydrous and presumably immobilized state, and is motionally averaged to an axially-symmetric spectrum of breadth ~110 ppm upon dispersion in 50 wt % H_2O at 20°C. In the presence of 1.2 mol ratio of UO_2^{2+}, the spectrum is very similar to that of the anhydrous sample (see Fig. 2). The slight decrease in spectral linewidth to ~130 ppm can be attributed to some small amplitude motion. The ^{13}C data therefore suggests that binding of UO_2^{2+} to the lipid molecule completely inhibits the fast axial diffusion. Thus, except for some small amplitude motion, the carbonyl group is virtually immobilized. Although this does not exclude the possibility that the motion of the phosphodiester group, or the head group as a whole, may be completely decoupled from the rest of the lipid molecule, this type of motion has not been observed. In addition, the strong binding of four lipid molecules to one uranyl ion is likely to prevent any large amplitude motion. Therefore, we conclude that the change in ^{31}P spectrum is the result of direct binding of UO_2^{2+} to the phosphodiester group leading to inhibition of axial diffusion.

In gel phase bilayers the binding of UO_2^{2+} leads to a decrease of the molecular area to ~44 Å^2 (Furuya et al., 1976) with a concomitant decrease in hydrocarbon chain tilt (Parsegian et al., 1981). In monolayers (see Fig. 7) the same phenomenon is observed for DPPC in the liquid-

condensed phase, the molecular area decreasing to 45 Å^2 (Shah, 1968). The cross-sectional area of two hydrocarbon chains in the all-*trans* conformation, however, is smaller with ~41 Å^2. As the chain tilt is removed upon UO_2^{2+}-binding, the effective area for the acyl chains becomes larger despite the decrease in molecular surface area, and this facilitates *trans-gauche* isomerization. The ^{2}H-NMR spectra support his notion as ~40% *gauche* isomers are used for the stimulation of the ^{2}H spectra. In liquid-expanded monolayers, which correspond to the liquid crystalline in bilayers, the condensation effect is even larger. In this case, the molecular area is reduced by 10 to 57 Å^2. This condensation at high temperature is also evident in the ^{2}H spectra where at 60°C the splitting $\Delta\nu_Q$ is 34 kHz for the UO_2^{2+}/DPPC complex as compared with 23 kHz for pure L_α DPPC.

These structural changes, induced by the binding of UO_2^{2+} to the DPPC headgroup, also lead to changes in the dynamic characteristics of the lipid molecule. The most dramatic effect is the absence of axial diffusion as evident from the observation of rigid ^{13}C and ^{31}P spectra as discussed above. However, the ^{2}H-NMR spectra show another interesting aspect of molecular motion. In contrast to the ^{13}C- and ^{31}P-NMR spectra which are virtually unchanged from 20° to 45°C, the ^{2}H quadrupole NMR spectrum transforms from a flat top spectrum to a nearly $\eta = 1$ spectrum at 40°C (see Fig. 3). For an immobilized methylene deuteron, the deuterium quadrupole spectrum is nearly a Pake pattern with the perpendicular edge splitting being 127 kHz. Depending on the mode and rate of molecular motion, various patterns can be observed when the deuteron jump begins to move. For example, a fast axial rotation, or discrete jump motion of C_3 or higher symmetry will result in an axially symmetric spectrum such as the narrow component shown in Fig. 3. On the other hand, a fast two-site tetrahedral jump motion with equal populations will generate a totally asymmetric $\eta = 1$ spectrum such as that observed in N-palmitoylgalactosylceramide (Huang et al., 1980). The broad component observed in the UO_2^{2+}/DPPC complex is nearly a perfect $\eta = 1$ spectrum and can be simulated with a three-site tetrahedral jump motion with population ratios of 0.63:0.30:0.07 and an exchange rate of $\sim 3.1 \times 10^7$ s^{-1} for k_{12}. The $\eta = 1$ lineshape observed in the ^{2}H spectrum of N-palmitoylgalactosylceramide has been interpreted as due to the three-bond crank-shaft motion, which is identical to a kink diffusional motion. This type of motion requires the presence of large fractions of *gauche* isomers, and is not generally observed when axial diffusion is present as is the case in pure phospholipids. For this reason the ^{2}H spectrum of *sn*-2(4,4-d$_2$)DPPE has been simulated with rotational diffusion motion of a nearly all-*trans* acyl chain (Blume et al., 1982).

The observation of an asymmetric ^{2}H spectrum for the UO_2^{2+}/DPPC complex is consistent with the fact that the available surface area per molecule is larger than the cross-sectional area of two all-*trans* acyl chains and with the experimental finding that the chains are not tilted with respect to the bilayer normal. Because rotational diffusion cannot occur and the acyl chains are physically separated, thermal energy can be used for *trans-gauche* isomerization as in the case of the glycolipids. At higher temperatures the UO_2^{2+}/DPPC complex undergoes a transition to a liquid crystalline phase characterized by fast axial diffusion of the molecules and rapid *trans-gauche* isomerization in the acyl chains. However, this liquid crystalline phase is, due to the bound UO_2^{2+} ions, considerably more condensed than the liquid crystalline phase of pure DPPC. It is comparable to liquid crystalline phases of other lipids with strong headgroup interactions such as N-palmitoylgalactosylceramide or to dipalmitoylphosphatidylethanolamine, where quadrupole splittings of more than 30 kHz were also observed.

We should also mention that we have observed three splittings of $\Delta\nu_Q$ = 28, 19, and 12.5 kHz in the deuterium spectrum of 1.2 UO_2^{2+}/sn-1, 2(2,2-^{2}H) DPPC 50 wt % H_2O sample at 60°C (T.-h. Huang and R. G. Griffin, manuscript submitted for publication), suggesting that in the liquid crystalline state the backbone conformation of the UO_2^{2+}/DPPC complex resembles that of the pure phospholipids (Seelig and Seelig, 1980).

CONCLUSIONS

The application of different NMR techniques in combination with monolayer and DCS experiments has provided a detailed picture of the structural and dynamical changes occurring in DPPC bilayers when UO_2^{2+}-cations are bound to the headgroups.

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